REMARKS

1. Applicants hereby submit the following:

[ ] a paper copy of a "Sequence Listing", complying with §1.821(c), to be incorporated into the specification as directed above;

[XX] an amendment to the paper copy of the "Sequence Listing" submitted on January 10, 2001, the amendment being in the form of substitute sheets. SEQ ID NOs:11-13 were added. SEQ ID NO:11 is shown in Figure 1 (P. fluorescens) as filed; SEQ ID NO:12 is shown in the specification on page 55, lines 17-18, and SEQ ID NO:13 is shown on page 57, lines 15-16. The only other changes made were in the general information sections <130>, <140;141> and <150;151>;

[XX] the Sequence Listing in computer readable form, complying with §1.821(e) and §1.824, including, if an amendment to the paper copy is submitted, all previously submitted data with the amendment incorporated therein;

[ ] pursuant to §1.821(e), reference is made to the computer readable form filed on , in USSN , which presents the identical Sequence information, the use of which is now requested, in lieu of submitting a new computer readable form; and/or

[ ] a substitute computer readable form to replace one found to be damaged or unreadable.

[XX] 2. The description and claims have been amended to comply with §1.821(d).

3. The undersigned attorney or agent hereby states as follows:

(a) this submission is not believed to include new matter [§1.821(g)];

(b) the contents of the paper copy (as amended, if applicable) and the computer readable form of the Sequence Listing, are believed to be the same [§1.821(f) and §1.825(b)];

(c) if the paper copy has been amended, the amendment is believed to be supported by the specification and is not believed to include new matter [§1.825(a)]; and

(d) if the computer readable form submitted herewith is a substitute for a form found upon receipt by the PTO to be damaged or unreadable, that the substitute data is believed to be identical to that originally filed [§1.825(d)].

4. Under U.S. rules, each sequence must be classified in <213> as an "Artificial Sequence", a sequence of "Unknown" origin, or a sequence originating in a particular organism, identified by its scientific name.

Neither the rules nor the MPEP clarify the nature of the relationship which must exist between a listed sequence and an organism for that organism to be identified as the origin of the sequence under <213>.

Hence, counsel may choose to identify a listed sequence as associated with a particular organism even though that sequence does not occur in nature by itself in that organism (it may be, e.g., an epitopic fragment of a naturally

occurring protein, or a cDNA of a naturally occurring mRNA, or even a substitution mutant of a naturally occurring sequence). Hence, the identification of an organism in <213> should not be construed as an admission that the sequence *per se* occurs in nature in said organism.

Similarly, designation of a sequence as "artificial" should not be construed as a representation that the sequence has no association with any organism. For example, a primer or probe may be designated as "artificial" even though it is necessarily complementary to some target sequence, which may occur in nature. Or an "artificial" sequence may be a substitution mutant of a natural sequence, or a chimera of two or more natural sequences, or a cDNA (i.e., intron-free sequence) corresponding to an intron-containing gene, or otherwise a fragment of a natural sequence.

The Examiner should be able to judge the relationship of the enumerated sequences to natural sequences by giving full consideration to the specification, the art cited therein, any further art cited in an IDS, and the results of his or her sequence search against a database containing known natural sequences.

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page is captioned "Version with markings to show changes made".

Respectfully submitted,

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the specification:

Paragraph beginning at line 27 of page 47 has been amended as follows:

Figure 1 shows a comparison of the amino acid sequences of *CTH* of *Azotobacter vinelandii* as described herein and a pyridine nucleotide transhydrogenase from *P. fluorescens* (French *et al.*, 1997) (SEQ ID NO:11).

Paragraph beginning at line 13 of page 55 has been amended as follows:

A fraction of the concentrate was analysed by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). It showed two protein bands with the apparent M_r of approximately 38000 and 54000, respectively. Both protein bands were isolated from the gel for further analysis. The N-terminal sequence of the small SDS-PAGE protein band was determined by automated Edman degradation and was found to be M-K-V-Y-Y-D-K-D-A-D-L-S-I-I-Q-S-K-K-V-A-I (SEQ ID NO:12). A similarity search through all databases available on the world wide web using the BLASTP 1.4.11 program (Altschul *et al.*, 1990) showed 76% identity and 100% similarity to the $NADP^+$-specific acetohydroxy acid isomeroreductase from *Rhizobium meliloti*, which has a M_r of 36518. The cells used in this work

were grown in minimal medium while the growth medium used in the earlier purification of transhydrogenase is unknown. Since the enzyme is involved in *de novo* synthesis of branched chain amino acids, absence of the protein in the earlier purification procedure could be explained by the use of a rich growth medium.

Paragraph beginning at line 4 of page 57 has been amended as follows:

Analysis of the amino acid sequence. The deduced amino acid sequence of CTH_p is listed in SEQ ID NO:2. The protein has a M_r of 51345, as determined by the Compute pI/Mw program on the world wide web (Bjellqvist *et al.*, 1993). This is slightly lower than the M_r of 54000 expected from the migration position in SDS-PAGE. Furthermore, the amino acid composition fitted well with earlier determinations (Voordouw *et al.*, (1980); Middleditch *et al.*, 1972). A similarity search through all databases available on the world wide web using the BLASTP 1.4.11 program (Altschul *et al.*, 1990) showed 84% identity and 91% similarity to the protein sequence of the soluble pyridine nucleotide transhydrogenase from *P. fluorescens* (French *et al.*, 1997). As observed for the *P. fluorescens* protein, CTHp had a similarity of up to 52% to the pyridine nucleotide-disulphide oxidoreductases, e.g.

dihydrolipoamide dehydrogenases, glutathione reductases and mercuric ion reductases. These are FAD flavoproteins which contain an active site with the consensus pattern G-G-X-C-(L/I/V/A)-X-G-C-(L/I/V/M)-P (SEQ ID NO:13) where the two cysteines are involved in the transfer of reducing equivalents from the FAD cofactor to the substrate (Kuriyan *et al.*, 1991). A similar pattern is observed in the two soluble transhydrogenases from amino acid 41 to 51 in Figure 1, but one of the cysteine residues is missing. Hence, the characteristic active site, that is observed in other flavoproteins, is absent in the soluble transhydrogenases. A high similarity of 75% was observed to an unknown dehydrogenase from *E. coli*. The protein is encoded by *udhA*, which is located next to *oxyR* in the chromosome of the bacterium. Since *oxyR* is a part of the positive regulation of genes involved in the defence against oxidative damage (Tao *et al.*, 1989), *udhA* could have a role in this process. Defence against oxidative stress is dependent on consumption of NADPH through glutathione reductase in most organisms and the high similarity between the two soluble transhydrogenases and *udhA* could indicate a physiological role of the three enzymes in synthesis of NADPH when the cell is subjected to oxidative stress. Two βαβ or Rossman fold motifs (G-X-G-X-X-G) forming the FAD and NAD(P) binding sites are observed at identical

locations in both soluble transhydrogenases. This fits well with earlier findings where two different binding sites for nicotinamide nucleotides were identified in the soluble transhydrogenase of *P. aeruginosa* (Höjeberg *et al.*, 1976) and is also supported by a proposed model of the allosteric regulation of soluble transhydrogenases (Rydström *et al.*, 1987). One site binds both NADP(H) and NAD(H) and constitutes the active site where transfer of reducing equivalents between the two cofactor systems occurs through a simple ping-pong mechanism (Widmer & Kaplan, 1977). The second site binds only to NADP(H) and is involved in allosteric regulation of the enzyme activity by NADP(H) and 2'-nucleotides. By sequence homology studies of several NAD^+-binding enzymes it has been found that the NAD(H) binding site of these enzymes in addition to the Rossman fold motif consists of an acidic residue (aspartate or glutamate) 18-19 residues upstream of the last glycine residue in the Rossman fold and a glycine residue 13 residues further upstream of the acidic residue (Olausson *et al.*, 1995). This is observed for the Rossman motif located from amino acid 12 to 17 which indicates that NAD(H) binds to this site and thus, that it is the active site of the enzyme.